

10. (amended) A method for assessing the binding of a test compound to a membrane containing the  $I_{K_r}$  channel using a radioligand compound of Formula I, [ $^{35}\text{S}$ ]-radiolabeled (+)-N-[1'-(6-cyano-1,2,3,4-tetrahydro-2(R)-naphthalenyl)-3,4-dihydro-4(R)-hydroxyspiro[2H-1-benzopyran-2,4'-piperidin]-6-yl]-methanesulfonamide, comprising the steps of:

- 1) preparing solutions of the test compound at 5 or more different concentrations, a solution of control vehicle and a solution of (+)-N-[1'-(6-cyano-1,2,3,4-tetrahydro-2(R)-naphthalenyl)-3,4-dihydro-4(R)-hydroxyspiro[2H-1-benzopyran-2,4'-piperidin]-6-yl]-methanesulfonamide (compound of Formula II) in a solvent;
- 2) mixing the radioligand compound of Formula I with the membrane containing the  $I_{K_r}$  channel diluted with an assay buffer to form a membrane/radioligand mixture of known concentration;
- 3) incubating a quantity of the membrane/radioligand mixture with the solution of test compound, control vehicle or compound of Formula II, as recited in Step 1, for a set time period at a temperature range of between about 40°C and about 37°C to give a mixture of membrane bound with the radioligand and the test compound, the control vehicle or the compound of Formula II;
- 4) isolating from the incubated mixture the membrane bound with the radioligand and the test compound, the membrane bound with the radioligand and with the control vehicle or the membrane bound with the radioligand and the compound of Formula II;
- 5) measuring the radioactivity of the isolated membrane bound with the radioligand and the test compound, the membrane bound with the radioligand and with the control vehicle or the membrane bound with the radioligand and the compound of Formula II;
- 6) repeating steps 3 through 5 with the test compound at each concentration, the solution of control vehicle and the solution of the compound of Formula II, as recited in Step 1;
- 7) calculating the  $\text{IC}_{50}$  corresponding to the measured radioactivity of: 1) the membrane bound with the radioligand and each concentration of the test compound, 2) the membrane bound with the radioligand and with the control vehicle, and 3) the membrane bound with the radioligand and the compound of Formula II, to assess the binding of the test compound to the membrane.

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